# METHEMOGLOBIN AS A POSSIBLE ANTIDOTE IN CYANIDE POISONING

-- FINAL REPORT --

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December 28, 1987



Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Project Order No. 87PP7826

Department of the Army
Letterman Army Medical Center
Presidio of San Francisco, California 94129-6700

SECURITY CLASSIFICATION OF THIS PAGE

# REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

| | |
|---|---|
| 1a. REPORT SECURITY CLASSIFICATION<br>Unclassified | 1b. RESTRICTIVE MARKINGS |
| 2a. SECURITY CLASSIFICATION AUTHORITY | 3. DISTRIBUTION/AVAILABILITY OF REPORT<br>Approved for public release; distribution unlimited. |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE | |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S) | 5. MONITORING ORGANIZATION REPORT NUMBER(S) |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Department of the Army<br>Letterman Army Medical Center<br>6b. OFFICE SYMBOL (If applicable)<br>HSHH-CI | 7a. NAME OF MONITORING ORGANIZATION |
| 6c. ADDRESS (City, State, and ZIP Code)<br>Presidio of San Francisco, CA 94129-6700 | 7b. ADDRESS (City, State, and ZIP Code) |
| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION<br>U.S. Army Medical Research and Development Command<br>8b. OFFICE SYMBOL (If applicable) | 9. PROCUREMENT INSTR' 'MENT IDENTIFICATION NUMBER<br>Project Order No. 87PP7826 |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick, Frederick, Maryland 21701-5012 | 10. SOURCE OF FUNDING NUMBERS<br>PROGRAM ELEMENT NO. 62734A<br>PROJECT NO. 3M162734 A875<br>TASK NO. AA<br>WORK UNIT ACCESSION NO. 370 |

11. TITLE (Include Security Classification)

Methemoglobin as a Possible Antidote in Cyanide Poisoning.

12. PERSONAL AUTHOR(S)
Garry W. Boswell

| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| Final | FROM 3/1/87 TO 12/15/87 | 1987 Dec 28 | 21 |

16. SUPPLEMENTARY NOTATION

| 17. COSATI CODES | | | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) |
|---|---|---|---|
| FIELD | GROUP | SUB-GROUP | |
| 06 | 15 | | Methemoglobin, Antidote, Pharmacokinetics, Cyanide. |
| 06 | 11 | | |

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of this study was to determine the effectiveness of exogenously prepared methemoglobin (MetHb) as an antidote to cyanide poisoning and to determine the pharmacokinetic parameters of MetHb when given by intravenous (iv) injection to rats. The effects of the administration of MetHb prepared in vitro were evaluated in Sprague-Dawley rats given increasing doses of potassium cyanide (KCN). Median lethal dose ($LD_{50}$) studies were conducted using KCN given by intraperitoneal (ip) injection (in 0.3- to 0.5-ml volumes) and then 2 min later administering doses of either 1000, 1500, or 2500 mg/kg of MetHb iv via tail vein injection. Control rats received an equivalent volume of saline. The resulting $LD_{50}$ values were $7.4 \pm 1.1$, $11.7 \pm 1.1$, $13.9 \pm 1.0$, and $14.2 \pm 1.0$ mg/kg (mean $\pm$ SD) for control (no MetHb), 1000, 1500, and 2500 mg/kg dose groups, respectively. Additional groups of rats were given 1000, 1500, and 2500 mg/kg MetHb only and submitted for necropsy. The gross finding of darkened kidneys was present
(continued on attached sheet)

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|---|---|
| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT<br>☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS | 21. ABSTRACT SECURITY CLASSIFICATION<br>UNCLASSIFIED |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>Mary Frances Bostian | 22b. TELEPHONE (Include Area Code)<br>301-663-7325<br>22c. OFFICE SYMBOL<br>SGRD-RMI-S |

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

Project Order No. 87PP7826
DD Form 1473, Item 19 Continued

in both dose groups, but became consistent and more prominent in the 2500-mg/kg dose group. Evidence of pathologic changes was not present in other organs. Single-dose pharmacokinetic studies were conducted using iv doses of 1600 and 2500 mg/kg. The elimination half-life was similar in both doses (62.6 min), but the volume of distribution (95.3 $\pm$ 7.2 and 126.3 $\pm$ 5.2 ml/kg, mean $\pm$ SEM) and clearance (1.06 $\pm$ 0.05 and 1.49 $\pm$ 0.12 ml/min/kg) were significantly different ($p < 0.05$) for the 1600 and 2500 mg/kg doses, respectively. From these data we conclude that although MetHb is cleared from the vascular system rapidly, it may be an effective and nontoxic cyanide antidote for doses up to twice the control $LD_{50}$.

**SUMMARY**

The purpose of this study was to determine the effectiveness of exogeneously prepared methemoglobin (MetHb) as an antidote to cyanide poisoning and to determine the pharmacokinetic parameters of MetHb when given by intravenous (iv) injection to rats. The effects of the administration of MetHb prepared in vitro were evaluated in Sprague-Dawley rats given increasing doses of potassium cyanide (KCN). Median lethal dose ($LD_{50}$) studies were conducted using KCN given by intraperitoneal (ip) injection (in 0.3- to 0.5-ml volumes) and then 2 min later administering doses of either 1000, 1500, or 2500 mg/kg of MetHb iv via tail vein injection. Control rats received an equivalent volume of saline. The resulting $LD_{50}$ values were 7.4 ± 1.1, 11.7 ± 1.1, 13.9 ± 1.0, and 14.2 ± 1.0 mg/kg (mean ± SD) for control (no MetHb), 1000-, 1500-, and 2500-mg/kg dose groups, respectively. Additional groups of rats were given 1000, 1500, and 2500 mg/kg MetHb only and submitted for necropsy. The gross finding of darkened kidneys was present in both dose groups, but became consistent and more prominent in the 2500-mg/kg dose group. Evidence of pathologic changes was not present in other organs. Single-dose pharmacokinetic studies were conducted using iv doses of 1600 and 2500 mg/kg MetHb. The elimination half-life was similar in both doses (62.6 min), but the volume of distribution (95.3 ± 7.2 and 126.3 ± 5.2 ml/kg, mean ± SEM) and clearance (1.1 ± 0.1 and 1.5 ± 0.1 ml/min/kg) were significantly different ($p < 0.05$ for the 1600- and 2500-mg/kg doses, respectively. From these data we conclude that although MetHb is cleared from the vascular system rapidly, it may be an effective and nontoxic cyanide antidote for doses up to twice the control $LD_{50}$.

## FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

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## INTRODUCTION

Many cyanide compounds are very toxic, and individuals may be exposed to these compounds from both natural and man-made sources. Many foods, some drugs, and tobacco smoke produce enough cyanide to cause concern (1,2). Cyanide also presents a hazard to soldiers in the form of chemical warfare agents. Many nations still maintain stocks of cyanide compounds in their arsenals. Another important source of cyanide exposure is through accidental fires (3). One study showed that of 36 fire survivors who had clinical evidence of smoke inhalation, carboxyhemoglobin and cyanide levels were elevated in all survivors (4).

The current standard of practice for treatment of cyanide intoxication in the United States includes the use of intravenous (iv) sodium nitrite and sodium thiosulfate solutions (1). The rationale is based on work of Chen and Rose (5) and others (6) who showed that: 1) nitrites convert hemoglobin to methemoglobin (MetHb), which then binds the cyanide ion and reduces the toxic effects; and 2) thiosulfate serves as a source of sulfur for the conversion of the cyanide to the less toxic thiocyanate. Recently this proposed mechanism for nitrite detoxification has been questioned, primarily on the grounds of the slow rate of MetHb formation and the resulting length of time required for effective levels of MetHb to be produced (6). However, when nitrites are administered prior to cyanide exposure, MetHb formation does play a role in the detoxification mechanism (7).

The effectiveness of MetHb in the treatment of cyanide poisoning may be exploited by the in vitro preparation of MetHb solutions. Exogeneously administered MetHb has been shown to be an effective antidote for cyanide intoxication in rats (8). This study demonstrated a 62% increase in the survival rate of rats given oral sodium cyanide; however, the dose of cyanide used in this study was below the median lethal dose ($LD_{50}$) for cyanide. In many cases of cyanide intoxication, the dose of cyanide is unknown but is possibly greater than the $LD_{50}$. The current study was undertaken to explore the use of exogeneously administered MetHb with doses of cyanide that exceed the $LD_{50}$ and to determine the pharmacokinetic profile of exogeneously administered MetHb in rats.

## METHODS

Potassium cyanide solutions were prepared fresh in 0.9% saline for each experiment from reagent-grade chemicals (J.T. Baker Chemical Co, Phillipsberg, NJ) and maintained in sealed containers. Other reagents used were reagent grade (USP) or better.

MetHb solutions were prepared from stroma-free hemoglobin (SFH) solutions obtained either from outdated human blood by the method of Rabiner et al. (9) or from Travenol Laboratories, Inc, Morton Grove, IL. The SFH was combined with a 10% solution of potassium ferrocyanide (5 ml/450 ml of SFH), and the mixture was allowed to stand overnight at 4°C. This mixture was dialyzed with 0.9% saline to remove any residual potassium ferrocyanide or reaction products, concentrated to a MetHb concentration of 11 g/dl to 23 g/dl filtered through a 0.22-micron filter (Millistat, Millipore Corp, Bedford, MA) into 150-ml plasma transfer packs, stored at -80°C until needed, and then thawed and stored at 4°C between uses. The MetHb concentration was determined by the method of Evelyn and Malloy (10).

Male Sprague-Dawley rats (average weight approximately 450 g) were maintained on a 12-hr light-dark cycle and were allowed food and water ad libitum. The median lethal dose studies were conducted using four groups of four rats that received intraperitoneal (ip) injections with increasing doses of potassium cyanide (in 0.3- to 0.5-ml volumes). Two minutes later, the test dose of MetHb was given by rapid (<60 sec) intravenous (iv) injection into a tail vein. The rats were returned to their cages and observed for 24 hr. Deaths within the first 24 hr were considered to be related directly to cyanide. At the end of 24 hr, surviving rats were euthanized by ip injection of sodium pentobarbital. MetHb doses of 1000, 1500, and 2500 mg/kg were studied. Median lethal dose values were calculated using the method of moving averages (11).

Control groups of rats received injections of either 1500 mg/kg of MetHb, 2500 mg/kg of MetHb, or 2500 mg/kg of human serum albumin (Armour Pharmaceutical Co., Kankakee, IL). At the end of 24 hr, these rats were euthanized and submitted for necropsy. Immediately upon death, blood samples were taken and gross necropsies performed. Brain, lungs, liver, heart, urinary bladder, gastrointestinal tract, kidneys, adrenals, spleen, stifle (femur/tibia), and sternum were collected and fixed with 10% buffered formalin. After routine imbedding of samples in paraffin, representative sections were cut at approximately 7 microns and stained with hematoxylin and eosin.

For pharmacokinetic studies, a carotid artery catheter was surgically implanted and the rats were allowed to recover 3 days before the MetHb was injected. The dose of MetHb (1600 mg/kg or 2500 mg/kg) was injected via tail vein in less than 1 min and timed 0.3-ml blood samples were collected from the carotid artery catheter. The blood samples were stored at $4^{O}C$ until analyzed. No more than 10 samples were withdrawn from any single animal. Catheter patency was maintained by flushing between sample collections with 0.3 ml heparinized saline (10 U/ml). Twenty-four hours after the injection of MetHb, the rats were euthanized and submitted for necropsy. The time versus MetHb concentration data obtained were analyzed by nonlinear regression analysis. The resulting pharmacokinetic parameters for both doses were compared using the Student's t test. All statistical procedures were performed using the BMDP statistical programs (12).

MetHb concentrations were measured in whole-blood samples spectrophotometrically at 620 nm in 1-cm quartz cuvettes. A 40-microliter aliquot of whole blood was added to 2 ml of 10 mM sodium borate buffer (pH approximately 9.1) containing 0.1% Flaminox solution (Fisher Scientific Co., Springfield, NJ). During the course of this study, Triton X-100 (J.T. Baker Chemical Co., Phillipsberg, NJ) was substituted for Flaminox in the buffer solution because Flaminox was discontinued by the manufacturer. The sample was mixed by inversion and the absorbance measured on a model 8451A Diode Array spectrophotometer (Hewlett-Packard, Santa Clara, CA). Standard curve solutions were prepared by addition of 0.0, 0.025, 0.050, 0.075, 0.1, 0.2, 0.3, 0.4, and 0.5 ml of the previously prepared MetHb solution to the borate buffer for a final volume of 1 ml. A 40-microliter aliquot of each concentration was added to 2 ml of borate buffer and the absorbance determined as above. Blood standards were prepared from pooled rat blood by addition of MetHb to give concentrations as previously noted.

## RESULTS AND DISCUSSION

### Methemoglobin Preparation

Three lots of MetHb were prepared for this study. The first lot was obtained using SFH prepared from outdated human packed red blood cells and the other two lots were obtained from SFH prepared under contract. The method of preparation of the MetHb was the same for each lot and produced essentially identical products containing 120 mEq/l $Na^+$, 0.05 mEq/l $K^+$, a pH of 7.25-7.3, an osmolality of 282-305 mOsm/l, and MetHb concentrations of 11, 20, and 23 g/dl. The final MetHb solution contained no non-heme protein (measured by isoelectric focusing techniques). The higher concentrations were prepared to allow higher doses of MetHb to be administered without exceeding 25% of the estimated rat blood volume. MetHb solutions containing greater than about 25 g/dl were difficult to sterilize by filtration; thus, this concentration became the upper limit for useful preparations.

### Median Lethal Dose Studies

As shown in Fig. 1, there was an increase in the $LD_{50}$ with increasing doses of MetHb, from 7.4 $\pm$ 1.1 mg/kg (mean $\pm$ SD) in the control animals (no MetHb) to 11.7 $\pm$ 1.1, 13.9 $\pm$ 1.0, and 14.2 $\pm$ 1.0 with doses of 1000, 1500, and 2500 mg/kg, respectively. The $LD_{50}$ values for all MetHb doses were significantly different ($p < 0.05$) from the $LD_{50}$ values for the controls. The $LD_{50}$ for the 1500-mg/kg group was significantly different from that of the 1000-mg/kg group but not from that of the 2500-mg/kg group ($p < 0.05$). However, when the administration of the MetHb was delayed beyond about 3 min after the cyanide dose, the rats died before the MetHb could affect survival. There is a clear asymptotic trend in the $LD_{50}$ with increasing MetHb doses, suggesting 2500 mg/kg approaches a maximally effective dose. Consequently, the higher doses initially proposed were not studied. At the $LD_{50}$ doses of KCN, the molar ratio of potassium cyanide ions to MetHb binding sites decreases from 11.7 to 5.7 for the 1000 and 2500 mg/kg doses, respectively. It is noteworthy that doubling the relative amount of MetHb gives only 21% improvement in the effectiveness of the antidote. Therefore, doses exceeding 1000 mg/kg provided limited additional protection against cyanide intoxication.

### Pathology

Three groups of nonsurgically treated rats and three groups of rats with surgically implanted catheters were submitted for pathology studies. The nonsurgically treated rats received 1500 mg/kg (eight rats, Group 1) or 2500 mg/kg (five rats, Group 2) of MetHb. Rats with surgically implanted catheters received 1000 mg/kg MetHB (five rats, Group 3), 1500 mg/kg MetHb (13 rats, Group 4), 2500 mg/kg MetHb (eight rats, Group 5), or 2500 mg/kg human serum albumin (five rats, Group 6). Gross and microscopic examinations were performed on all animals, and blood for analysis was obtained from four rats in Group 2. Four rats in Group 1 and five rats in Group 5 showed gross mild, diffuse, bilateral kidney discoloration, which was not detectable microscopically. All rats in Groups 1, 2, and 5 had grossly discolored kidneys and three rats in Group 1 had black speckling of the kidneys. Microscopically, rats in Groups 2 and 5 had protein droplets in the cytosol of the proximal convoluted tubules consisting of 1- to 2-micron, round, eosinophilic structures, which were tinctorially consistent with MetHb. Two rats in Group 2 and all rats in Group 5 had protein globules in the cortical tubular lumina. These globules consisted of occasional 5- to 7-micron, amorphous, amphophilic, irregularly round structures. One rat had a mild nephrosis that consisted of variable numbers of eosinophilic, hypertrophied cortical tubular cells, with a few necrotic cells that were sloughing into the tubular lumina. This rat had a significantly elevated blood urea nitrogen (BUN) level. All rats tested had a slight elevation of the BUN level. Other changes seen in other organs were mild, incidental, and unrelated to dosage with MetHb.

### Analytical Development

Standard curves based on MetHb absorbance at 620 nm were linear over the range 0-110 mg/dl. Regression of the absorbance versus MetHb concentration demonstrated a small day-to-day deviation in the standard curve regression line (Table I). As can be seen in Fig. 2, there was excellent correlation between the amount of MetHb added to rat blood samples and that measured at 620 nm using buffer standard curves. The between-day reproducibility of spiked rat blood samples is shown in Table II.

**Pharmacokinetics**

As seen in Fig. 3, the MetHb was essentially completely cleared from the blood by 4 hr in both the 1600-mg/kg (Fig. 3A) and the 2500 mg/kg (Fig. 3B) doses. No evidence of nonlinear elimination was seen. The data from each dose fitted well a one-compartment open model with bolus input and produced the pharmacokinetic parameters shown in Table III. The elimination rate for both doses was essentially the same. The volume of distribution ($V_d$) for MetHb approximates the extracellular water volume (13). The greater total-body clearance of MetHb at the higher dose primarily reflects the greater calculated $V_d$. Although quantitative urine collection was not done, MetHb appeared in the urine in less than 15 min after the dose was given. The elimination mechanism for MetHb is presumed to be similar to that for unbound hemoglobin. This mechanism involves the reticuloendothelial system, liver, and kidneys and is dose-dependent (14). However, the present data suggest that with MetHb apparent first-order elimination occurs in rats at doses of 2500 mg/kg or less. Because the current data suggest that relatively little accumulation would be expected with MetHb administered in the expected dosing applications, the originally proposed continuous infusion and multiple-dose studies were not conducted.

In summary, the present study demonstrated the value of in vitro prepared MetHb as an antidote to cyanide intoxication with cyanide doses that are twice the $LD_{50}$ in control animals. There appeared to be minimal acute pathological effects resulting from intravenous MetHb administration, but the longer-term toxicities have not yet been evaluated. Spectrophotometric measurements of MetHb at 620 nm provided a fast and accurate method of determining whole-blood levels of MetHb. Following single intravenous doses of 1600 and 2500 mg/kg, the elimination of MetHb appeared to be first order with a terminal-phase half-life of 62.6 min. In addition, the volume of distribution at these doses approximated that of extracellular water.

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**TABLE I. Between-Day Standard Curve Reproducibility**

| Standard Curve No. | Intercept[a] | Slope |
|---|---|---|
| 1 | 0.00342 | 0.003141 |
| 2 | 0.000013 | 0.003208 |
| 3 | -0.00222 | 0.00318 |
| 4 | 0.004743 | 0.003128 |
| 5 | 0.000529 | 0.00323 |
| 6 | 0.000185 | 0.003359 |
| 7 | 0.001194 | 0.003126 |
| 8 | 0.004888 | 0.003218 |
| 9 | 0.005132 | 0.003047 |
| 10 | 0.000292 | 0.003 |
| 11 | 0.002440 | 0.003501 |
| 12 | 0.002375 | 0.003239 |
| 13 | -0.00038 | 0.00322 |
| 14 | -0.00064 | 0.0034 |
| 15 | 0.00071 | 0.00315 |
| 16 | 0.00065 | 0.00322 |
| 17 | 0.0056 | 0.00318 |
| 18 | 0.00233 | 0.00319 |
| 19 | -0.00011 | 0.00339 |
| 20 | -0.00114 | 0.00327 |
| 21 | 0.00118 | 0.00317 |
| 22 | -0.00021 | 0.00318 |
| 23 | 0.00247 | 0.00328 |
| 24 | -0.00095 | 0.0033 |
| 25 | 0.00164 | 0.00329 |
| 26 | 0.00324 | 0.00325 |
| 27 | -0.00158 | 0.00329 |
| 28 | 0.00157 | 0.00353 |
| 29 | -0.00006 | 0.0034 |
| 30 | 0.0047 | 0.00352 |
| Mean: | 0.001400 | 0.003253 |
| Standard deviation: | 0.002099 | 0.000126 |

[a] The intercept and slope of the least-squares regression line fitted to buffered standard curves data for MetHb standards.

**TABLE II. Between-Day Assay Precision and Accuracy for Prepared Methemoglobin Standards[a]**

| Experiment No. | Sample Concentration (mg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.00 | 5.46 | 10.92 | 16.39 | 21.85 | 43.70 | 65.55 | 87.40 | 109.25 |
| 1 | 0.23 | 5.61 | 11.19 | 17.35 | 21.97 | | | | |
| 2 | -0.52 | 6.69 | 11.38 | 16.69 | 22.11 | | | | |
| 3 | 0.43 | 5.60 | 11.09 | 16.65 | 22.58 | | | | |
| 4 | 1.51 | 4.55 | 10.25 | 17.12 | 22.93 | | | | |
| 5 | 0.44 | 6.07 | 10.58 | 16.10 | 23.16 | | | | |
| 6 | 0.60 | 5.52 | 10.86 | 16.66 | 22.72 | | | | |
| 7 | 0.24 | 5.84 | 11.19 | 16.49 | 22.59 | | | | |
| 8 | 0.42 | 5.73 | 10.86 | 16.76 | 22.58 | | | | |
| 9 | 0.09 | 5.64 | 11.64 | 16.88 | 22.10 | | | | |
| 10 | 0.88 | 6.14 | 11.09 | 16.20 | 20.89 | 45.85 | 65.62 | 84.64 | 110.94 |
| 11 | 0.67 | 5.95 | 11.54 | 16.48 | 21.97 | 44.20 | 64.16 | 86.95 | 110.32 |
| 12 | 0.17 | 5.94 | 11.08 | 16.43 | 22.60 | 43.55 | 65.53 | 88.46 | 108.49 |
| 13 | 2.46 | 5.16 | 10.60 | 16.27 | 21.67 | 44.11 | 64.73 | 87.18 | 110.07 |
| 14 | 0.82 | 5.74 | 10.96 | 16.25 | 22.32 | 43.78 | 65.42 | 88.11 | 108.85 |
| 15 | -0.12 | 5.17 | 10.60 | 15.58 | 22.57 | 46.15 | 67.39 | 87.91 | 107.01 |
| 16 | 0.57 | 5.75 | 11.11 | 16.21 | 21.86 | 45.07 | 66.53 | 84.64 | 110.53 |
| 17 | 0.43 | 5.60 | 11.37 | 17.65 | 24.29 | 44.66 | 68.88 | 88.32 | 104.17 |
| 18 | 2.13 | 7.69 | 12.73 | 18.32 | 23.91 | 48.65 | 69.29 | 95.43 | 119.76 |
| 19 | 0.64 | | | | 20.82 | 43.77 | 65.65 | 87.88 | 108.98 |
| 20 | 0.22 | | | | 21.75 | 43.39 | 67.01 | 84.69 | 110.68 |
| 21 | -0.48 | | | | 21.05 | 44.31 | 68.51 | 85.27 | 109.09 |
| 22 | | | | | 22.34 | 43.11 | 65.66 | 87.01 | 109.64 |
| 23 | 1.47 | | | | 21.70 | 41.42 | 65.83 | 86.93 | 110.40 |
| Mean: | 0.60 | 5.80 | 11.12 | 16.67 | 22.28 | 44.43 | 66.44 | 87.39 | 109.92 |
| Standard deviation: | 0.721 | 0.632 | 0.525 | 0.616 | 0.823 | 1.624 | 1.507 | 2.607 | 3.225 |
| Coefficient of variation: | 119.20 | 10.90 | 4.72 | 3.70 | 3.69 | 3.65 | 2.27 | 2.98 | 2.93 |

[a] Between-day precision and accuracy of measured MetHb concentrations in prepared standards using a wavelength of 620 nm.

**TABLE III. Methemoglobin Pharmacokinetic Parameters[a]**

| Rat No. | $C_o$ (mg/ml) | $V_d$ (ml/kg) | $K_{el}$ (1/min) | $T_{1/2}$ (min) | Dose (mg/kg) | Weight (kg) | Clearance (ml/min/kg) |
|---|---|---|---|---|---|---|---|
| | | | **1600-mg/kg Dose** | | | | |
| 71 | 13.06 | 123.79 | 0.008531 | 81.25 | 1616.9 | 0.500 | 1.06 |
| 79 | 19.11 | 85.27 | 0.013423 | 51.64 | 1629.49 | 0.590 | 1.14 |
| 80 | 16.96 | 96.64 | 0.011002 | 63.00 | 1638.75 | 0.560 | 1.06 |
| 83 | 21.01 | 77.98 | 0.012143 | 57.08 | 1638.75 | 0.680 | 0.95 |
| 84 | 15.16 | 107.11 | 0.011673 | 59.38 | 1624.25 | 0.565 | 1.25 |
| 85 | 19.93 | 81.22 | 0.010929 | 63.42 | 1618.52 | 0.621 | 0.89 |
| Mean: | 17.54 | 95.33 | 0.01128 | 62.63 | 1627.78 | 0.586 | 1.06 |
| Standard deviation: | 2.54 | 16.08 | 0.00149 | 9.22 | 8.76 | 0.056 | 0.12 |
| | | | **2500-mg/kg Dose** | | | | |
| 41 | 22.25 | 111.49 | 0.011861 | 58.44 | 2480.31 | 0.508 | 1.32 |
| 42 | 22.34 | 111.44 | 0.015664 | 44.25 | 2489.63 | 0.482 | 1.75 |
| 43 | 22.79 | 109.93 | 0.011196 | 61.91 | 2505.40 | 0.463 | 1.23 |
| 48 | 21.79 | 114.26 | 0.010286 | 67.39 | 2489.80 | 0.490 | 1.18 |
| 49 | 24.75 | 101.58 | 0.015704 | 44.14 | 2514.29 | 0.525 | 1.60 |
| 50 | 22.18 | 112.93 | 0.007386 | 92.85 | 2504.35 | 0.575 | 0.83 |
| 89 | 16.21 | 155.70 | 0.007158 | 96.84 | 2523.51 | 0.319 | 1.11 |
| 91 | 20.17 | 134.39 | 0.015749 | 44.01 | 2710.19 | 0.314 | 2.12 |
| 93 | 16.75 | 149.28 | 0.011916 | 58.17 | 2501.09 | 0.366 | 1.78 |
| 96 | 19.06 | 131.31 | 0.011411 | 60.74 | 2502.94 | 0.340 | 1.50 |
| 97 | 17.79 | 139.08 | 0.008978 | 77.21 | 2473.84 | 0.344 | 1.25 |
| 99 | 17.45 | 144.24 | 0.01547 | 44.81 | 2516.72 | 0.329 | 2.23 |
| Mean: | 20.29 | 126.30 | 0.01190 | 62.67 | 2517.67 | 0.421 | 1.49 |
| Standard deviation: | 2.66 | 17.35 | 0.00304 | 17.71 | 59.73 | 0.090 | 0.40 |

[a] Pharmacokinetic parameters calculated from a one-compartment open model with bolus input. Individual rat MetHb concentration data were modeled using nonlinear regression analysis.

$C_o$ = initial concentration, $V_d$ = volume of distribution, $K_{el}$ = elimination rate constant, $T_{1/2}$ = terminal-phase half-life.

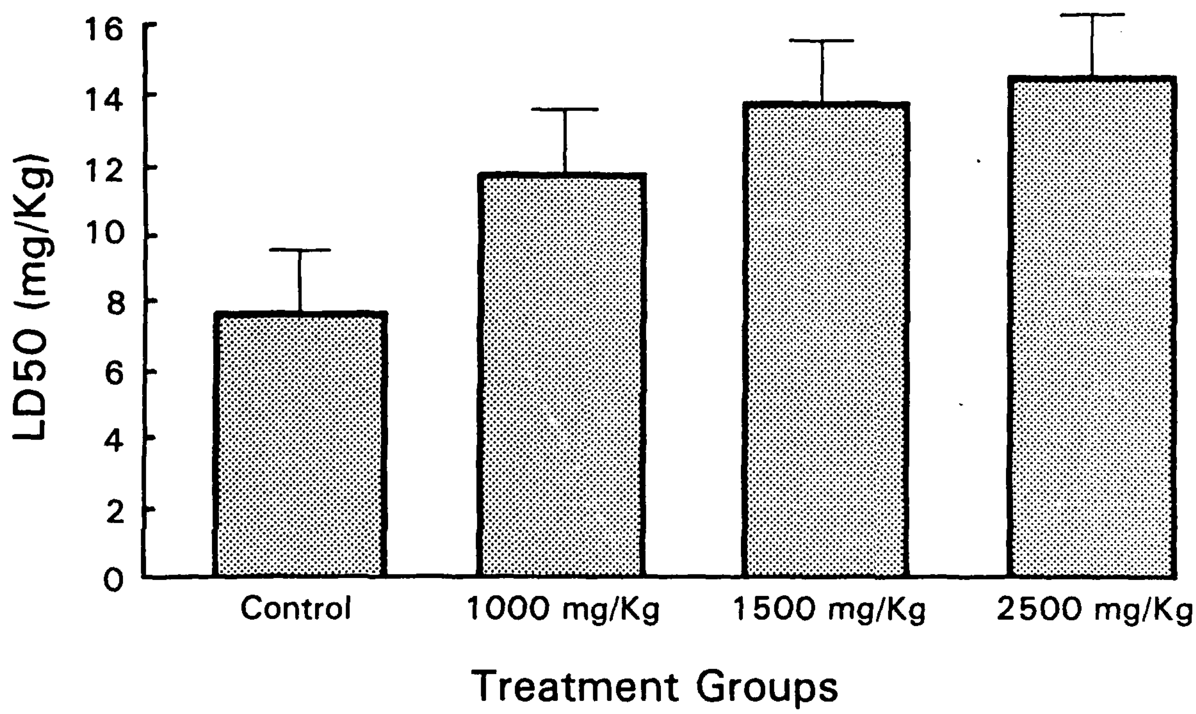



**Fig. 1.** Bar graph shows the increase of the median lethal dose in groups of rats given iv doses of 1000, 1500, and 2500 mg/kg methemoglobin 2 min following the ip administration of graduated doses of potassium cyanide.

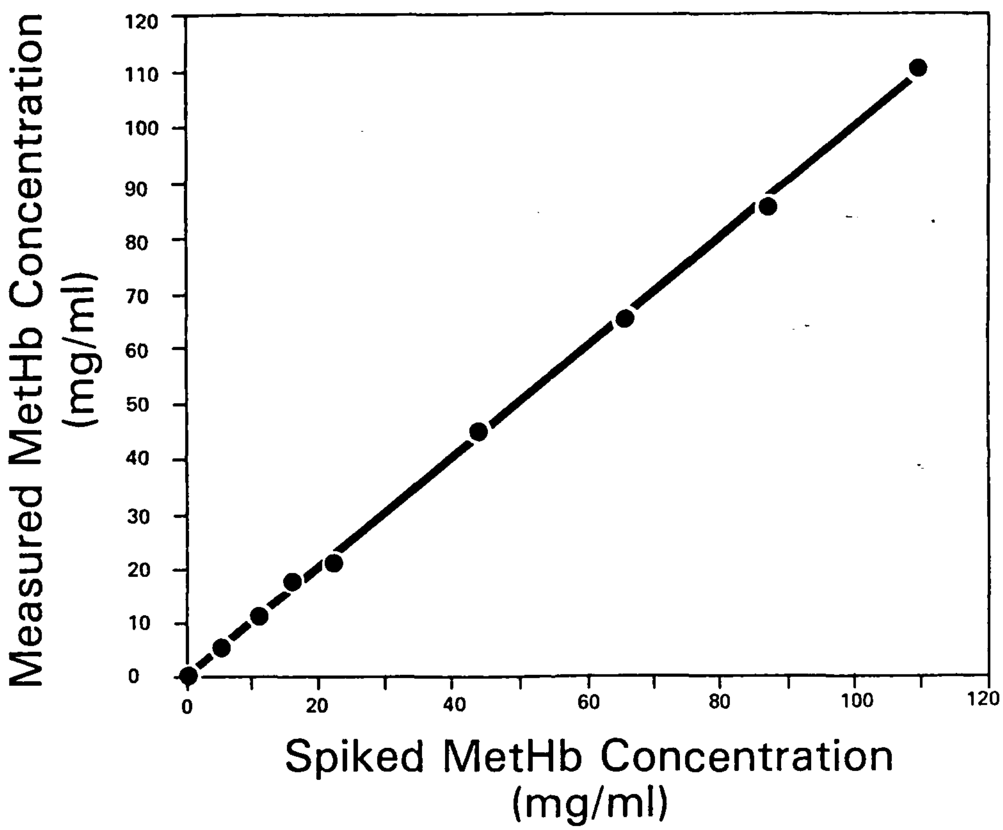
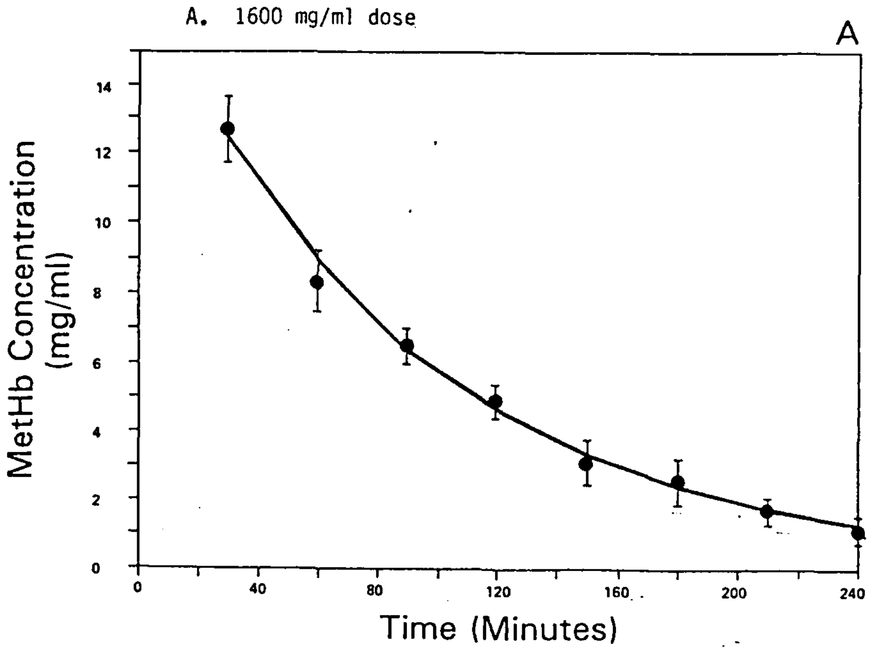



**Fig. 2.** Comparison of the concentration of MetHb measured in rat whole blood at 620 nm using buffer standard curves versus the actual prepared concentration. The prepared MetHb concentrations were 0.0, 5.5, 10.9, 16.4, 21.9, 43.7, 65.6, 87.4, and 109.3 mg/ml. Each point represents the mean of five samples. The standard error bars are too small to show.



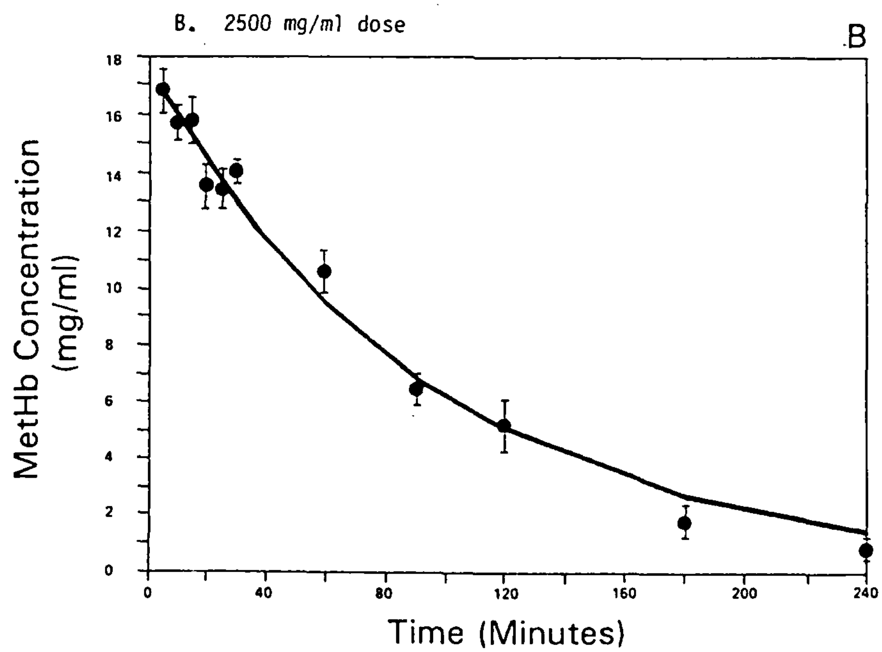




**Fig. 3.** Whole-blood concentrations of MetHb as a function of time following intravenous bolus dose of **(A)** 1600 mg/kg (mean $\pm$ SEM, n = 6) and **(B)** 2500 mg/kg (n = 12). The solid line represents the least-squares best-fit line of the data fitted to a one-compartment model.

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## PRESENTATIONS AND PUBLICATIONS

Presented in part in poster format at the First National Meeting of the American Association of Pharmaceutical Scientists, November 2-6, 1986, in Washington, DC.

Published as a journal article: Boswell GW, Brooks DE, Murray AJ, Doye AA, Disselhorst DF, Chin CL, Clifford CB. Exogenous methemoglobin as a cyanide antidote in rats. Pharmaceutical Research 1988;5:749-752.

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